Title – 20 words

Derivatization of Amino Acids in Human Plasma for Quantitation by Comprehensive Two Dimensional Gas Chromatography Time of Flight Mass Spectrometry

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Novel Aspect - 20 words

This is the first comparison of derivatization agents for amino acid analysis in human plasma using GCxGC TOFMS.

Introduction – 120 words

Metabolomics is an emerging field focused on analysis of low-molecular-weight compounds in complex biomatrices. These approaches are qualitative, quantitative, or a combination and can offer insights into mechanisms of disease or markers for diagnostics. NIST is developing a standard reference material, Metabolites in Human Plasma (SRM 1950), to serve as a common material to help researchers evaluate new techniques or platforms in this field. Both qualitative and quantitative information will be provided to the users of this material. Free amino acids have been identified as a class of metabolites to be quantified in the SRM. LC-MS/MS has been used to quantify amino acids in complex matrices and we are looking to develop an orthogonal method using GCxGC TOFMS.

Method – 120 words

SRM 1950 is a pooled human plasma material drawn from healthy adults. Extraction and derivatization of the amino acids is necessary to increase their volatility prior to analysis by GC. Three different derivatization agents were investigated: trimethyl silyl (MSTFA), tertbutyl silyl (MTBSTFA), and alkyl chloroformate. For the silyl derivatives a methanol extraction was performed which also precipitated the high molecular weight components. The methanol extract was dried down the reconstituted in derivatizing agents. The alkyl chloroformate reaction was performed in the aqueous matrix and the derivatized components extracted in organic solvent. The samples were separated using GCxGC and TOFMS was used for detection scanning m/z 40 – 800 at 20 - 200 scans/s throughout the chromatographic run.

Preliminary data – 300 words

There are advantages and disadvantages to each derivative. The silyl derivatives are more broadly applicable allowing multiple classes of metabolites to be analyzed simultaneously. However, in GC and to some degree in GCxGC these additional components, particularly glucose because of its high concentration, can cause interferences that complicate quantitation. The advantage of the GCxGC technique is the higher resolution and sensitivity which may be able to overcome the problem of interfering components. Thus far, twenty-two amino acids have been identified in SRM 1950 and are well resolved using silyl derivatization. The two different silylation agents appear to be complimentary, the retention order of the amino acids changes. For example, L-histidine is not resolved using MSTFA because it is too close to the large glucose peak however using MTBSTFA the peak further from glucose and is well resolved. The alkyl chloroformate derivatization is more selective for amino acids which

removes the major interfering peaks. This derivatization can be performed in aqueous media and is relatively quick compared to silylation. The disadvantage is that a more polar or a specific amine column (Rtx-35 or ZB-AAA) is necessary to resolve the species and very few other metabolites are seen. Depending on the specific alkyl chloroformate (e.g., propyl, buytl) some of the amino acids derivatives are either not volatile enough or not thermally stable and therefore cannot be analyzed using this derivatization. Quantitation of the amino acids in SRM 1950 was completed using each of the derivatizing agents. The results of quantitation as well as limits of detection, linear range, and relative standard deviations will be presented. Based on these comparisons the advantages and disadvantages of each of the derivatizing agents can be evaluated to pick the appropriate combination of agents to accurately quantify the largest number of amino acids in SRM 1950.